

A new dwarf chameleon, genus *Brookesia*, from the Marojejy massif in northern Madagascar

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Abstract

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We describe a new species of dwarf chameleon from the *Brookesia minima* species group. *Brookesia tedi* sp. n. occurs above 1300 m above sea level on the Marojejy massif in northeastern Madagascar. It is genetically sister to *B. peyrierasi*, a species occurring in lowlands around the Baie de Antongil, but is genetically strongly divergent from that species in both nuclear (c-mos) and mitochondrial (16S, ND2) genes, and morphologically in its smaller size and distinctly different hemipenis. It is the second species of the *B. minima* species group from Marojejy National Park, but is not known to occur in syntopy with the other species, *B. karchei*, due to elevational segregation.

Introduction

Chameleons, family Chamaeleonidae, are a highly diverse group of lizards, occurring across Africa and Madagascar, with a few species also present in southern Europe and Asia (Tilbury 2018). Body size within the family varies more than tenfold, from the giant species *Furcifer oustaleti* (Mocquard, 1894) at 284 mm maximum snout-vent length (SVL) to the dwarf species *Brookesia micra* Glaw, Köhler, Townsend & Vences, 2012 whose maximum SVL is ca 20 mm snout-vent length (Glaw et al. 2012a; Villeneuve 2017). According to current phylogenetic understanding of the evolution of these lizards (Tolley et al. 2013), chameleons were ancestrally semi-terrestrial, probably roosting above the ground on small twigs and branches at night, and relatively small-bodied, and sub-

sequently evolved fully arboreal habits. Members of the genus *Brookesia* are generally semi-terrestrial (most are terrestrial by day but roosting above the ground at night) and are all comparatively small in size. Most members of the *Brookesia minima* species group are dramatically smaller than other *Brookesia*, and their diversity was long underappreciated because they are difficult to find and due to their miniaturisation and crypsis (Brygoo and Domergue 1975, Glaw et al. 1999). Genetic analysis and more intense fieldwork in northern Madagascar revealed additional species (Glaw et al. 2012a), as has been the trend in other miniaturised Malagasy vertebrate groups (e.g. Rakotoarison et al. 2017).

The *Brookesia minima* species group includes some of the smallest amniotes in the world (Glaw et al. 2012a; Villeneuve 2017). These miniaturised chameleons are dis-

tributed across the northern half of Madagascar, with one species also occurring in the Tsingy de Bemaraha Massif in the dry west (Schimmenti and Jesu 1996). Glaw et al. (2012a) recognised three clades within the *B. minima* group: Clade A (*B. desperata* Glaw, Köhler, Townsend & Vences, 2012; *B. tristis* Glaw, Köhler, Townsend & Vences, 2012; *B. micra*, *B. confidens* Glaw, Köhler, Townsend & Vences, 2012; *B. tuberculata* Mocquard, 1894) consisting of species distributed primarily in the far north of Madagascar, Clade B (*B. peyrierasi* Brygoo & Domergue, 1975 and *B. karchei* Brygoo, Blanc & Domergue, 1970) consisting of species distributed in northeastern Madagascar, and Clade C (*B. ramanantsoai* Brygoo & Domergue, 1975; *B. exarmata* Schimmenti & Jesu, 1996; *B. dentata* Mocquard, 1900; *B. sp.* ‘Betampona’) consisting of species distributed in central eastern and western Madagascar, with *B. minima* Boettger, 1893 from the Sambirano region in northwestern Madagascar occupying a position sister to Clade B. Some undescribed diversity is still known within this species group, however.

Brygoo et al. (1974) reported on four specimens (probably MNHN 1986.876–879, formerly all subsumed under the preliminary number 722/C) belonging to the *Brookesia minima* group that they collected at 1300 m above sea level (a.s.l.) in Marojejy, from the humid faces of ravines, where they were found in bushy foliage and among mosses. The colouration of the specimens was briefly described, but their overall morphology was not discussed. A further specimen collected from the region of Sambava (presumably MNHN 1974.251 or 1974.252) was also referred to the species. Brygoo and Domergue (1975) elected not to describe the species due to the lack of sufficient data. A specimen was later illustrated by Brygoo (1978), and he also reported therein that the specimens were closer to *B. tuberculata* than to *B. peyrierasi* in the morphology of their dorsolateral spines and the crests of the dorsal head. According to Brygoo (1978), G. Ramanantsoa had apparently assembled a collection of ca 40 individuals and indicated to Brygoo his intent to describe the species, which presented also hemipenial differences, but this description was never published.

On a recent expedition to Marojejy, we collected new material of this species, and can finally resolve the question of the identity of this population. In the present study we provide a formal taxonomic description of the new species, and provide genetic evidence for its close relationship to *B. peyrierasi*.

Materials and methods

Specimens were found via searching by torchlight at night and photographed the following morning. They were anaesthetised and subsequently euthanised by oral of lidocaine. Tissue samples were taken and stored in pure ethanol. They were then fixed with 90% ethanol and deposited in 75% ethanol for long-term storage. ZCMV and FGZC refer to field numbers of M. Vences and F.

Glaw, respectively. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM). Reference is also made to the Muséum National d’Histoire Naturelle, Paris, France (MNHN) and University of Michigan Museum of Zoology, Ann Arbor MI, USA (UMMZ).

Morphological analysis was conducted following Glaw et al. (2012a). Measurements were taken by MV to the nearest 0.1 mm using a digital calliper. The following measurements were taken: TL (total length); SVL (snout–vent length); TaL, tail length; HW, maximum head width; HH, maximum head height; ED, eye diameter; FORL, forelimb length.

For molecular analysis, we expanded the DNA sequence data set of Glaw et al. (2012a) for the two mitochondrial genes of NADH dehydrogenase subunit 2 (ND2) and 16S rRNA (16S), and the nuclear gene for oocyte maturation factor *mos* (*c-mos*). We added to the existing data sequences for the two individuals of the new species described herein, as well as one additional individual of *B. peyrierasi*. Genomic DNA was extracted and amplified using standard protocols as described in Glaw et al. (2012a). Gene fragments were amplified using the primers ND2F17 (5′-TGACAAAAAATTGCNCC-3′; Macey et al. 2000) and ALAR2 (5′-AAAATRTCTGRGTTGCATTCAG-3′; Macey et al. 1997) for ND2, 16SA-L (5′-CGCCTGTTATCAAAAACAT-3′) and 16S-BH (5′-CCGGTCTGAAGTCAGATCACGT-3′) for 16S (Palumbi et al. 1991), and CO8 (5′-GCTTGGTGTTCATAGACTGG-3′) and CO9 (5′-TTGGGAGCATCCAAAGTCTC-3′) for *c-mos* (Han et al. 2004). PCR products were purified with ExoSAPIT (Thermo Fisher Scientific, Waltham, MA, USA), sequenced on an automated DNA sequencer (ABI 3130 XL; Applied Biosystems). We used the software CodonCode Aligner (CodonCode Corporation) to check and correct errors in the DNA sequences based on the chromatograms, and to trim poor-quality stretches and primer sequences from the beginning and end of the sequences. We verified that none of the sequences of the protein-coding genes (ND2 and *c-mos*) contained stop codons, and retained hypervariable stretches in the 16S gene, as an exploratory analysis removing these yielded a similar tree topology. All new sequences obtained in the present study were submitted to GenBank (accession numbers MK452364–MK452366, MK457367–MK457369, and MK457452–MK457454); for accession numbers of previously published sequences, see Glaw et al. (2012a).

Alignment and analysis of sequences, separately for each marker, was performed in MEGA7 (Kumar et al. 2016). Sequences were aligned using the Clustal algorithm, and the most suitable substitution models determined under the Bayesian Information Criterion, implemented in MEGA7. Phylogenetic analyses were conducted under the Maximum Likelihood optimality criterion, with subtree-pruning-regrafting branch swapping (SPR level 5) and with 500 heuristic bootstrap replicates. Bootstrap values <50% (suggesting the respective node is recovered by less than half of the replicates) were omitted from the figures. We decid-

ed to analyse sequences for each marker separately rather than concatenating them because (1) a concordant signal of markers with different mode of inheritance (nuclear and mitochondrial) provides additional support for distinct evolutionary lineages under the genealogical concordance species criterion (Avise and Ball 1990), and (2) different sets of individuals have been sequenced for the three markers which would inflate a concatenated matrix with missing data, even for the two mitochondrial markers (for which the sequences in part refer to different individuals which we preferred not to combine to form chimera sequences).

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Results

Phylogenetic analysis of the nuclear (c-mos) and mitochondrial (ND2, 16S) markers yielded largely concordant results regarding the phylogenetic placement and molecular divergence of the specimens from Marojejy (Figs 1, 2). While deep relationships among clades of the *Brookesia minima* clade could not be reliably resolved in our single-marker analyses, the Marojejy specimens were in each case placed sister to *B. peyrierasi*, with moderate to strong support from bootstrap analysis (ND2: 99%; 16S: 84%; c-mos: 99%). However, the genetic differentiation between these two lineages were substantial: the Marojejy specimens differed from *B. peyrierasi* by 8.1–9.0% uncorrected pairwise distance (p-distance) in the 16S gene, 17.5–19.3% in the ND2 gene, and 1.7–2.7% in the usually very conserved c-mos gene. Their differentiation to the other species of the *B. minima* clade amounted to 10.8–17.5% for 16S, 26.1–29.2% for ND2, and 2.7–5.5% for c-mos.

Our genetic data confirm for the first time the presence of *B. peyrierasi* in a lowland site on the mainland opposite to its type locality, the islet of Nosy Mangabe (Fig. 3). One specimen from Ambodivoangy clustered with 98–100% bootstrap support with Nosy Mangabe specimens in the analyses of all three gene fragments (Figs 1, 2). Despite a substantial genetic differentiation between the Ambodivoangy and Nosy Mangabe specimens (3.2% in 16S, 7.9% in ND2, 0.7% in c-mos), at present there is no reason to consider them taxonomically distinct. This confirms *B. peyrierasi* as being a species distributed in lowlands of mainland Madagascar near Nosy Mangabe, and we here also assign a specimen from the Masoala peninsula (Fig. 4G, H) to this species based on morphology and biogeography, pending genetic confirmation. Two female specimens from Sambava MNHN 1974.251–252 that were

assigned to *B. peyrierasi* based on external morphology by Glaw et al. (1999) and a further uncollected juvenile from Sambava, depicted in Glaw and Vences (1994), are here omitted from further discussion because neither hemipenial nor molecular data are available from these specimens.

Morphological analysis revealed differences between the Marojejy specimens and *B. peyrierasi* in body size (*B. peyrierasi* being slightly larger; see diagnosis below and Table 1). We did not observe additional morphological differences, e.g. in the arrangements of scale rows dorsally on the head, and the two Marojejy individuals differed distinctly from each other in the appearance of their supraocular scales (Fig. 5). However, hemipenial morphology was drastically different between the Marojejy specimens and *B. peyrierasi* (Fig. 6), and the diagnostic characters were constant in all specimens of *B. peyrierasi* studied so far (Glaw et al. 1999) and in the two Marojejy individuals (see below for more details).

In addition, *B. peyrierasi* has so far been collected in lowland localities only (Fig. 3), whereas the two specimens of the Marojejy lineages came from forests ≥ 1300 m a.s.l. Taken together, the concordant and strong differences in mitochondrial and nuclear genes, body size, and hemipenial morphology provide unambiguous evidence for a species-level differentiation of the Marojejy lineage, and we therefore describe and name it as a new species as follows.

Taxonomy

Brookesia tedi sp. n.

<http://zoobank.org/8445F2DF-398F-4A6C-BB35-D322A36EDF7B>
Figures 5–7; Table 1

Remark. This species has been previously referred to as follows:

- B. "minima"* – Brygoo et al. (1974)
- B. minima* s. l. – Brygoo and Domergue (1975)
- B. aff. minima* – Brygoo (1978)
- B. cf. minima* – Glaw and Vences (1994)

Possibly also within the definition of *B. minima* by Raxworthy and Nussbaum (1995); see Referred specimens below.

Holotype. ZSM 438/2016 (ZCMV 15262), adult male, collected on 18 November 2016 in Camp ‘Simpoina’ (Camp 3) of Marojejy National Park (ca 14.4366S, 49.7434E, 1325 m a.s.l.), Sava Region, northeastern Madagascar by M.D. Scherz, A. Razafimanantsoa, A. Rakotoarison, M. Bletz, M. Vences, and J.H. Razafindralaibe.

Paratype. ZSM 439/2016 (ZCMV 15298), adult male, collected on 20 November 2016 from the same locality and by the same collectors as the holotype.

Referred specimens. Four specimens of *Brookesia* aff. *minima* (MNHN 1986.876–879, formerly all subsumed under the preliminary number 722/C, sex unidentified),

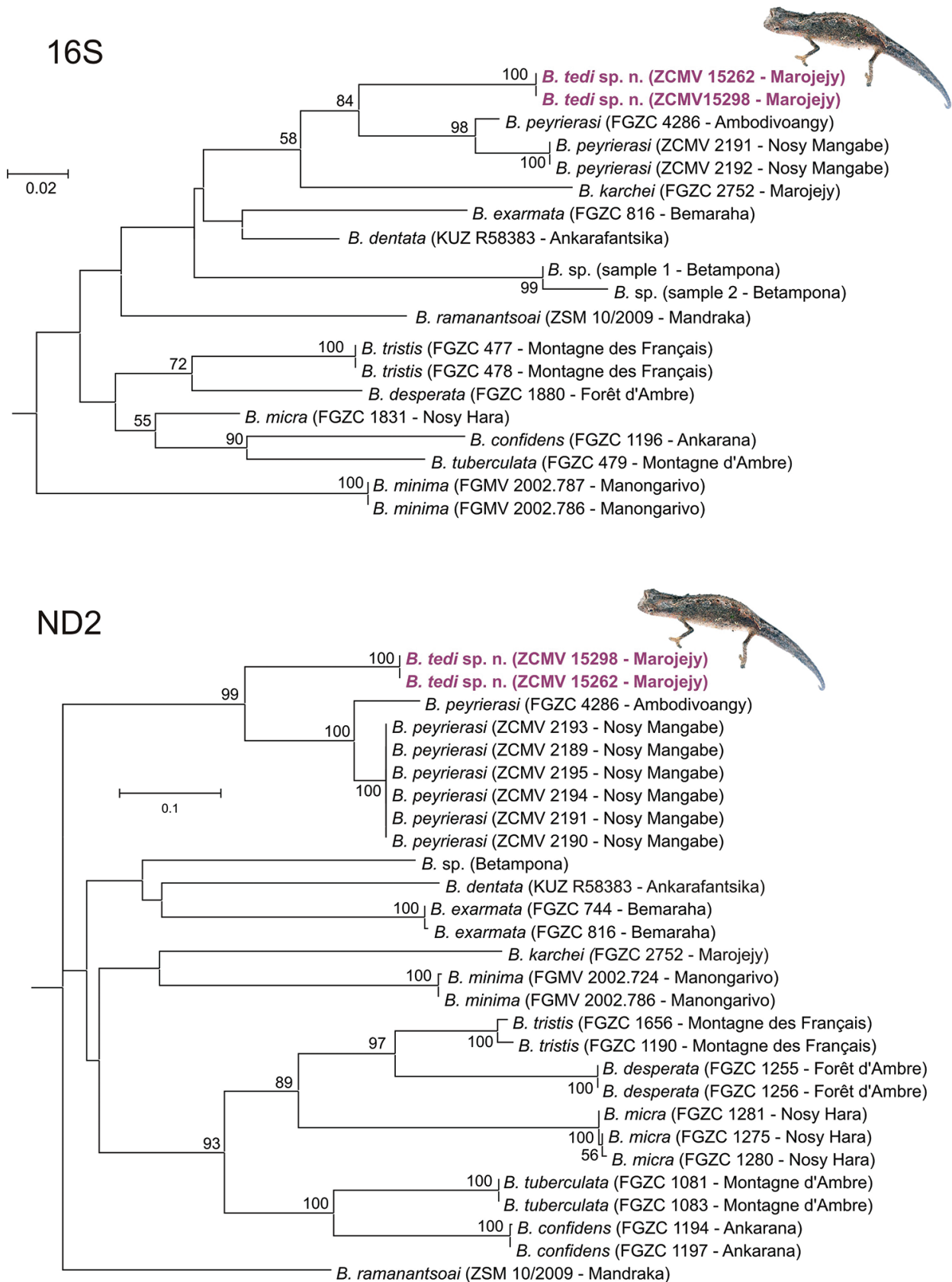


Figure 1. Molecular phylogenetic trees of species in the *Brookesia minima* group, based on sequences of the mitochondrial 16S (480 bp) and ND2 (574 bp) genes, inferred under the Maximum Likelihood optimality criterion, and the GTR+G (16S) and HKY+I+G (ND2) substitution models. Values at nodes are support values from a bootstrap analysis in percent (500 replicates) and are shown only if >50%. The two gene fragments were analysed separately and not concatenated because partly different samples were available for each of them. The trees were rooted with *Brookesia brygooi* (removed for better graphical representation).

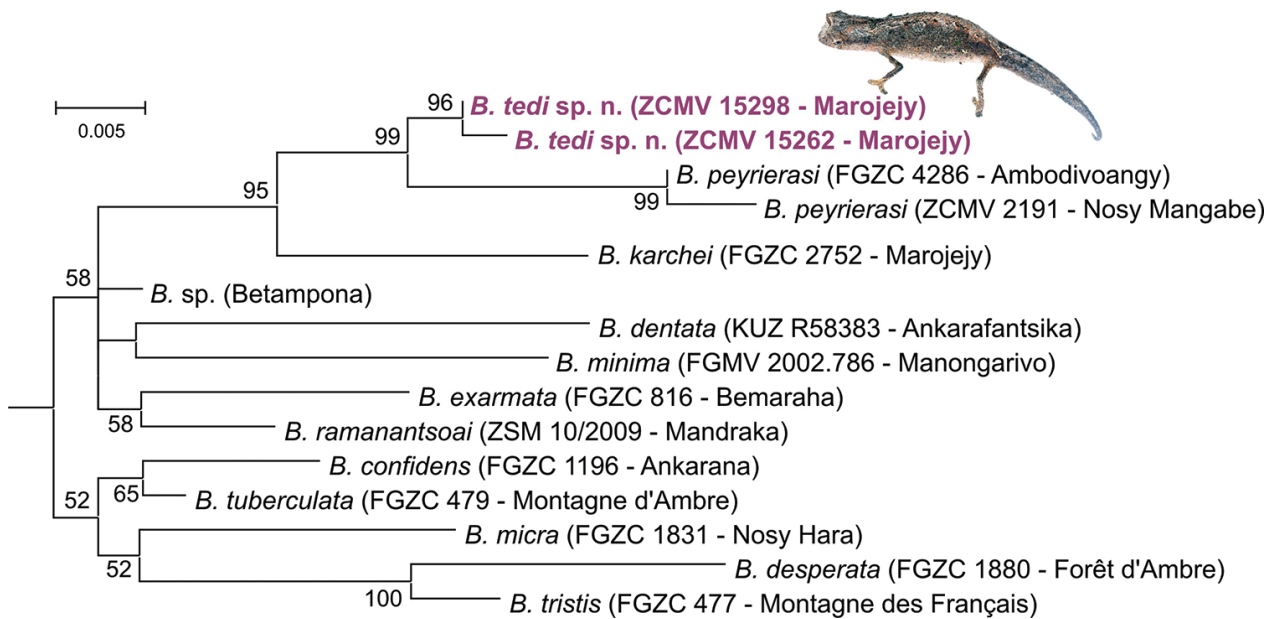


Figure 2. Molecular phylogeny of species in the *Brookesia minima* group, based on the nuclear c-mos gene (405 bp, no missing data) and inferred under the Maximum Likelihood optimality criterion (K2 substitution model). Values at nodes are support values from a bootstrap analysis in percent (500 replicates) and are only shown if >50%. The tree was rooted with *Brookesia brygooi* (removed for better graphical representation).

formerly housed in the Collection of the Institut Pasteur de Madagascar, were apparently collected ('event date') on 4 December 1972 at 'Marojezy' (=Marojejy), 1300 m a.s.l. according to the VertNet database (<http://portal.vertnet.org/search>, accessed 25 December 2018) and are probably the specimens referred to by Brygoo et al. (1974); we tentatively refer these specimens to this species without having examined them. The whereabouts of additional potential specimens (no. 722 and 723c) and of the ca 40 specimens collected by G. Ramanantsoa reported by Brygoo (1978) is unknown.

Specimens UMMZ 203615–203621 reported by Raxworthy and Nussbaum (1995) to be identical to *B. minima* may also be attributable to this species, but apparently come from lower elevation (200–800 m a.s.l.) and therefore cannot be referred to this species until they are genetically and morphologically investigated. In any case, given the morphological variation of *B. tedi* (see Discussion below) and the presence of one other species of the *B. minima* group at Marojejy (*B. karchei*), the identification of all these additional specimens as *B. tedi* will remain tentative unless molecular data can be obtained or fully diagnostic morphological or osteological characters defined in the future.

Diagnosis. A diminutive chameleon species assigned to the genus *Brookesia* on the basis of its small body size, short tail, crests of the head, dorsolateral spines and molecular relationships. *Brookesia tedi* sp. n. is morphologically characterised by the following unique suite of characters ($n = 2$ males): (1) SVL 15.3–18.2 mm; (2) TaL/SVL 0.74–0.92; (3) TL 29.5–31.7 mm; (4) HW/SVL

0.20–0.21; (5) 8–10 dorsolateral spines (when countable; sometimes spines are not or are only partly expressed, rendering them difficult to count); (6) distinct pelvic spine; (7) absence of lateral or dorsal spines on the tail; (8) presence of supraocular cone; (9) presence of supranasal cone; and (10) rather globular hemipenis with paired sets of small fleshy apical papillae.

Within the genus *Brookesia*, *B. tedi* sp. n. can easily be distinguished from all species that are not members of the *B. minima* species group based on its diminutive size (SVL 15.3–18.2 mm vs minimum 34 mm). Within the *B. minima* species group, it can be distinguished from males of *B. tristis* by longer relative tail length (TaL/SVL 0.74–0.92 vs 0.71–0.72), presence of supraocular cone (vs. absence); from males of *B. confidens* by slightly smaller body size (SVL 15.3–18.2 vs 18.3–20.1 mm), longer relative tail length (TaL/SVL 0.74–0.92 vs 0.60–0.70), presence of supraocular cone (vs absence), and globular hemipenes (vs tubular); from males of *B. micra* by longer relative tail length (TaL/SVL 0.74–0.92 vs 0.47–0.49), slightly narrower relative head width (HW/SVL 0.20–0.21 vs 0.23), slightly smaller relative head height (HH/SVL 0.16–0.18 vs 0.19–0.20), and globular hemipenes with paired fleshy apical papillae (vs tubular hemipenes with apical combs of papillae); from males of *B. desperata* by smaller body size (SVL 15.3–18.2 vs 25.0–26.7 mm) and total length (TL 29.5–31.7 vs 39.7–42.9 mm), longer relative tail length (TaL/SVL 0.74–0.92 vs 0.59–0.63), absence of lateral tail spines (vs presence), fewer dorsolateral spines (8–10 when countable vs 12–14), and hemipenes with fleshy papillae (vs single spines on strongly bilobed apex); from the male holotype of *B. exarmata* as reported by Schimmen-

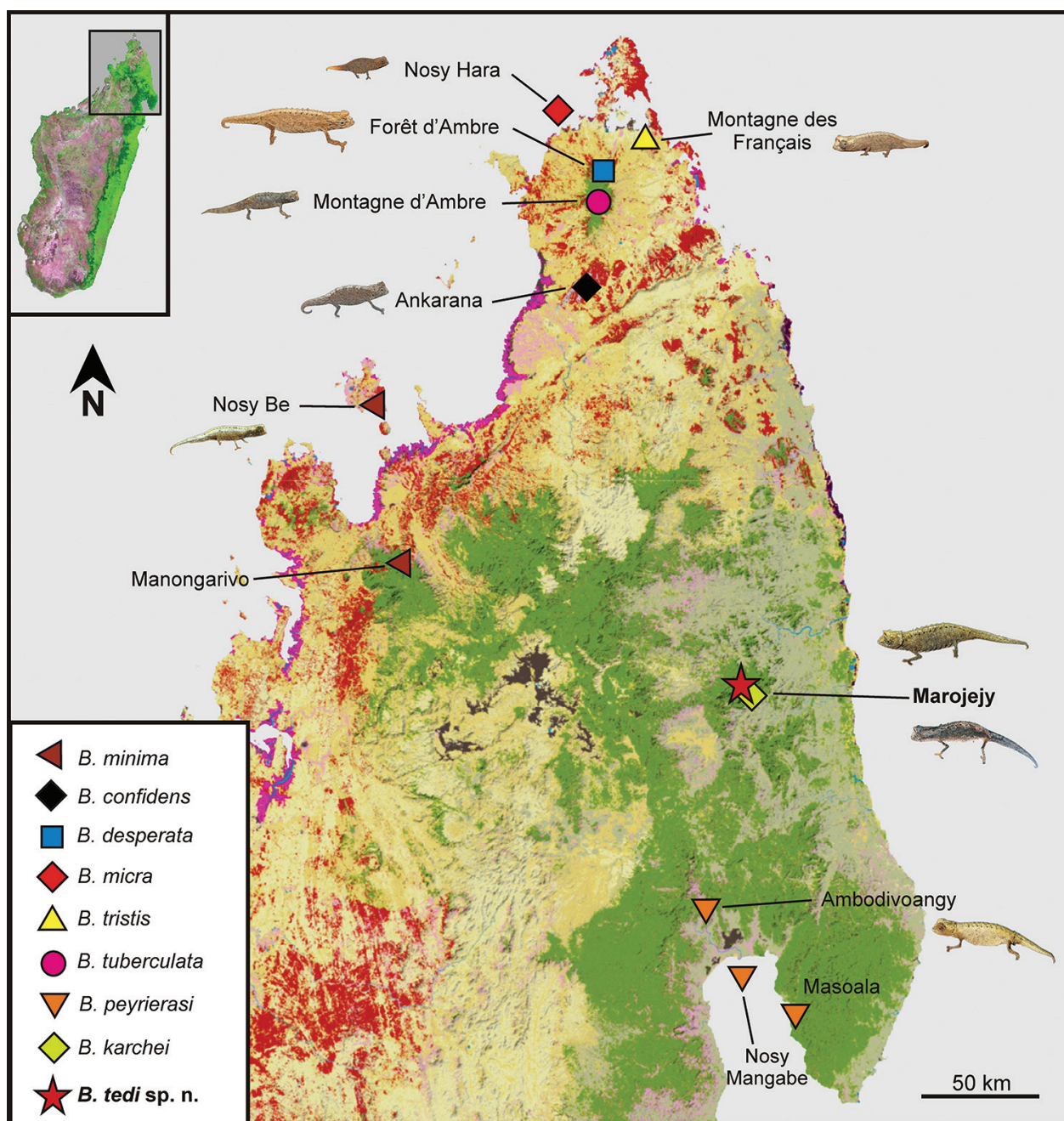


Figure 3. Map of northern Madagascar showing the localities of species of the *Brookesia minima* group in this region, only representing records verified by molecular data (except the *B. peyrierasi* record from Masoala and the *B. minima* record from Nosy Be). Note that *B. dentata*, *B. exarmata*, and *B. ramanantsoai* occur further south and are not included in the map. Red (dry forest) and green (rainforest) show remaining primary vegetation in 2003–2006, modified from the Madagascar Vegetation Mapping Project (<http://www.vegmad.org>); see the project for a key to the other colours and the vegetation types they indicate.

ti and Jesu (1996) by the presence of a supraocular cone (vs absence), slightly smaller relative head height (HH/SVL 0.16–0.18 vs 0.23), and slightly smaller total length (TL 29.5–31.7 vs 33.3 mm); from males of *B. minima* by slightly longer relative tail length (TaL/SVL 0.74–0.92 vs 0.65–0.73), slightly wider relative head width (HW/SVL 0.20–0.21 vs 0.16–0.19), presence of supraocular cone (vs absent), and presence of a distinct pelvic spine (vs absent

or indistinct); from males of *B. ramanantsoai* by smaller body size (SVL 15.3–18.2 vs 21.7 mm) and total length (TL 29.5–31.7 vs 39.0 mm), and wider relative head width (HW/SVL 0.20–0.21 vs 0.16); from males of *B. dentata* by smaller total length (TL 29.5–31.7 vs 43 mm) and presence of supraocular cone (vs absent); from females of *B. karchei* (as reported by Glaw et al. 2012a; no data from males are available) by longer relative tail length (TaL/SVL 0.74–

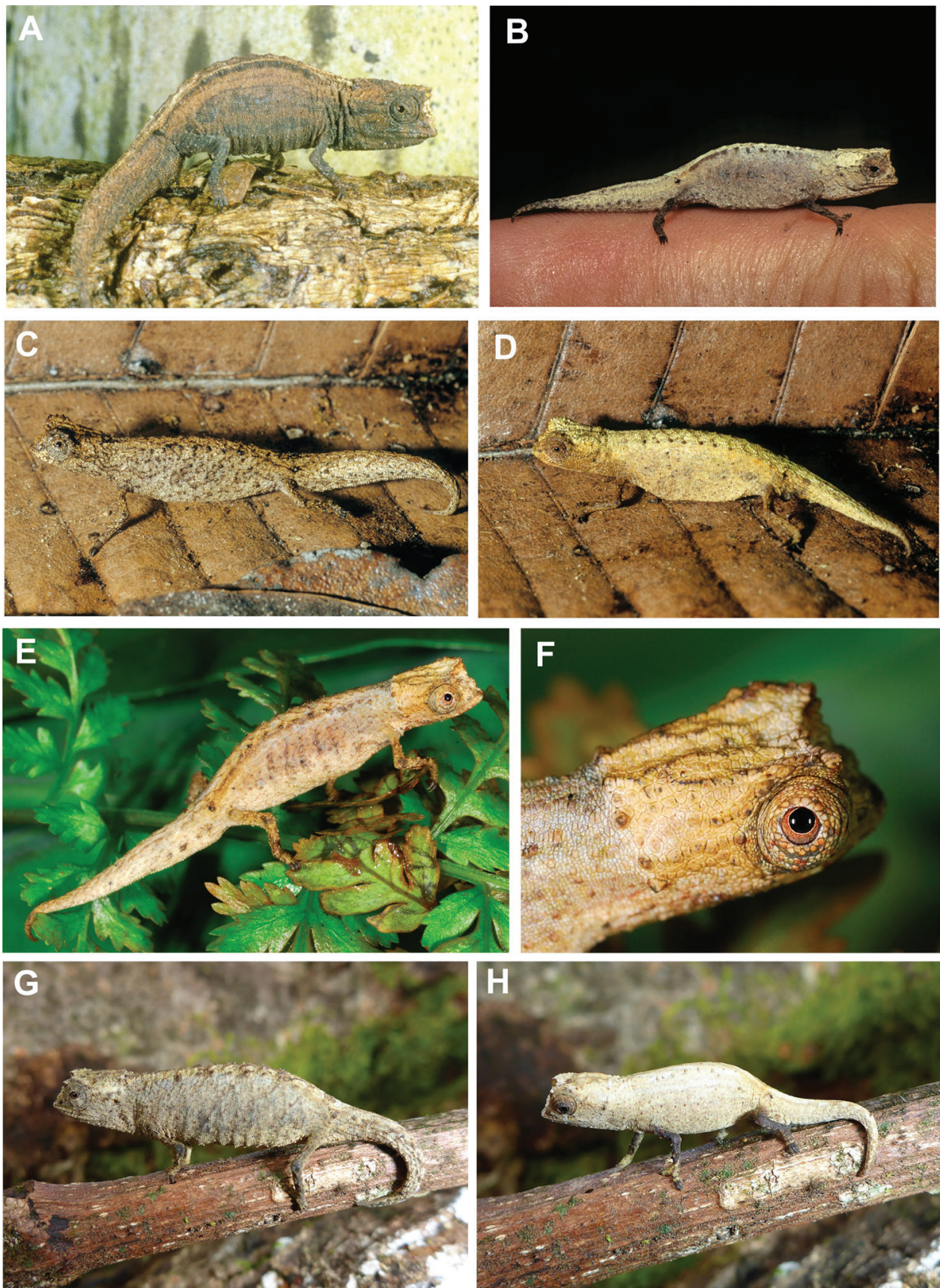


Figure 4. *Brookesia peyrierasi* in life A–D Specimens from the type locality Nosy Mangabe (specimen numbers unknown). E, F Specimen from Ambodivoangy, ZSM 447/2010 (FGZC 4286) G, H Specimens from Masoala (G, ZSM 252/2016 [FGZC 5429], H, specimen number unknown, species identity not confirmed genetically).



Figure 5. The holotype of *Brookesia tedi* sp. n. from Marojejy in lateral view (above), and dorsal (middle) and lateral (below) views of the heads of the types of *B. tedi* sp. n., in comparison with a representative male specimen of *B. peyrierasi* from the type locality Nosy Mangabe. The lateral view of the holotype (marked with an asterisk) has been mirrored to be in the same orientation as those of the other specimens. Scale bar: 1 mm.

0.92 vs 0.66), slightly wider relative head width (HW/SVL 0.20–0.21 vs 0.17), absence of lateral tail spines (vs presence), and fewer dorsolateral spines (8–10 when countable vs 13); and from males of *B. tuberculata* by smaller supraocular cone and globular hemipenis (vs tubular).

Molecular data clearly identify *B. peyrierasi* as the sister species of *B. tedi* sp. n. (Figs 1, 2); from males of *B. peyrierasi* the new species is distinguished by smaller body size

(SVL 15.3–18.2 vs 19.7–22.4 mm) and total length (TL 29.5–31.7 vs 34.2–39.8 mm), more distinct supraocular cone, and rather globular hemipenis lacking apical spines (vs tubular hemipenis with paired sets of apical spines; Fig. 6).

Description of the holotype. Adult male in good state of preservation (Figs 5, 7). Both hemipenes everted. Measurements in Table 1. Lateral crest on head weakly devel-

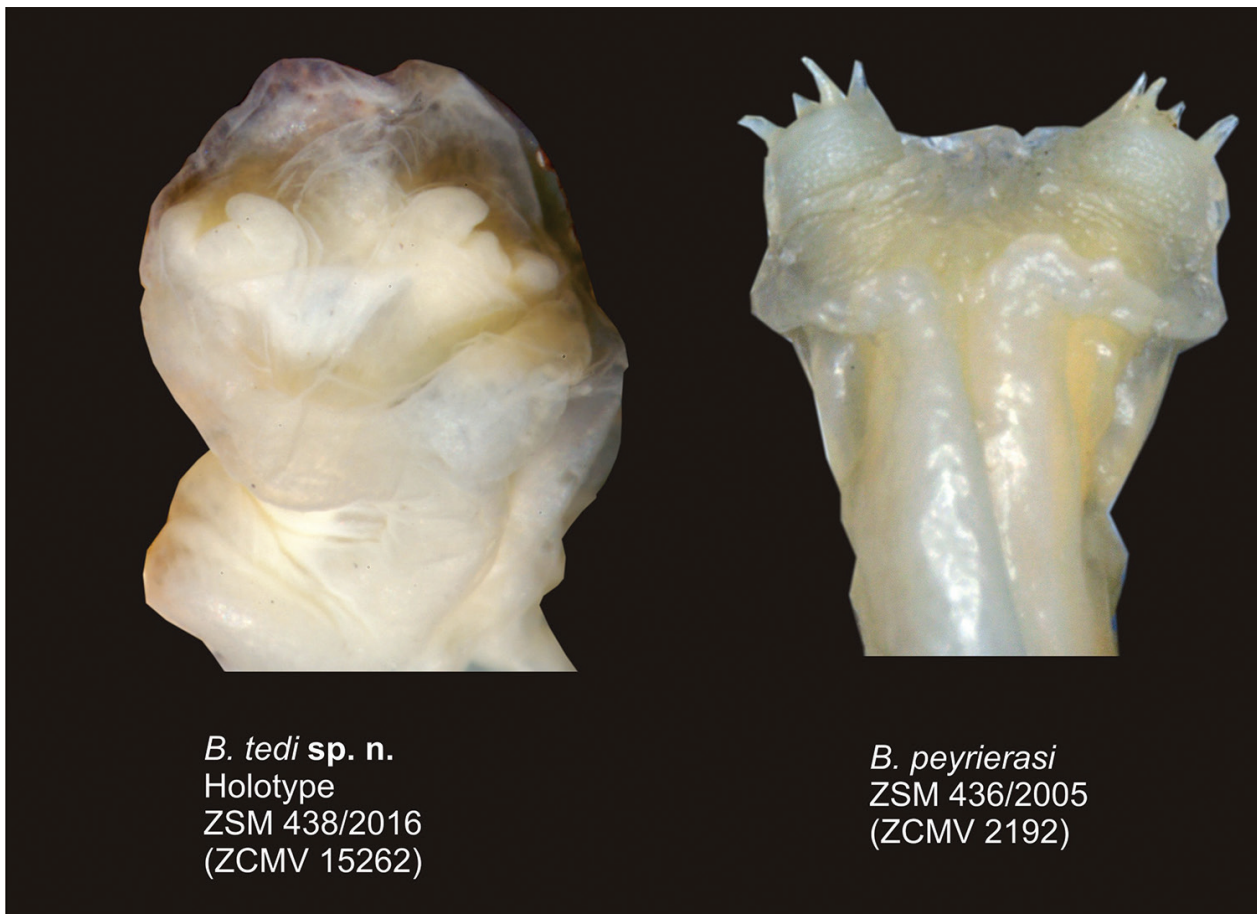


Figure 6. The preserved hemipenes of *Brookesia tedi* sp. n. and its sister species, *B. peyrierasi* in sulcal view. Not to scale. Note that the spines of *B. peyrierasi* are equally visible in asulcal view, and in all studied individuals of this species (see Glaw et al. 1999).

oped, barely recognizable; prominent orbital crests, and a transverse row of enlarged tubercles at the posterior edge of the head that separates the head from the body, no distinct posterior crest; a pair of curved parasagittal crests that start above the eyes and begin to converge before terminating at the transverse row of enlarged tubercles; depression between the eyes with short longitudinal median crest; three pointed tubercles on each side of posterior margin of head; scattered slightly enlarged tubercles on lateral surfaces of head; orbital crest denticulated; distinct supraocular cone present; supranasal cone small, not projecting beyond snout tip; head longer (5.1 mm) than wide (3.2 mm); chin and throat with evenly spaced distinctly enlarged tubercles. Dorsal surface of body without a vertebral ridge or keel; 10/8 (left/right) dorsolateral spines (pointed tubercles) form an almost complete longitudinal line on the body; posteriormost pointed dorsolateral tubercle being largest, above insertion point of hindlimb (the pelvic spine), very slightly projecting backwards; no dorsal pelvic shield in sacral area, but distinct pelvic spine; pointed dorsolateral tubercles almost equally spaced, except for first three tubercles on left side; dorsal surface of tail lacking distinctly enlarged tubercles; scattered enlarged tubercles laterally on anterior third of

tail; lateral surface of body with evenly spaced enlarged rounded tubercles; venter with distinctly enlarged rounded tubercles; scattered, distinctly pointed tubercles on limbs; no pointed tubercles around cloaca; longitudinal row of slightly enlarged tubercles lateral on anterior tail; no dorsal, lateral or ventral spines on tail; no enlarged tubercles on ventral surfaces of tail.

The hemipenis is rather globular (though not as globular as that of *B. minima*), short and broad, with a flattened apical end with a clear lip around its circumference (Fig. 6). The whole of the apex is somewhat tilted sulcally. A pair of structures emerge from the apical surface, each of which consists of three fleshy lobes, of which the middle lobe is the shortest. The truncus is smooth and lacks any trace of calyces.

In life, overall colouration light to dark brown, lighter ventrally, the dorsal head and dorsum down to the tail grey. Tubercles and patches of various other colours, including a number of nearly black spots, dot the flanks. Rectangular patches of grey invade the flank from the dorsum. The eye is rayed in shades of brown, with three especially light rays ventrally, the anterior two of which traverse across the upper and lower lips. A brown stripe bordered with cream traverses the supraocular cones.



Figure 7. Different views of the holotype of *Brookesia tedi* sp. n. (ZSM 438/2016; field number ZCMV 15262) in life.

The limbs are distinctly darker than the body. For further detail, see Figure 7. After two years in ethanol, the body colour is muted and more homogeneous. The rays on the eye and stripe between supraocular cones are still distinct, but the grey invading the flanks from the dorsum is less distinct.

Variation. For morphological measurements and proportions see Table 1. The male paratype ZSM 439/2016 differs from the male holotype by a far more prominent supraocular crest, formed by six large pointed tubercles, including a large supraocular cone; by three parallel dorsal crests between parasagittal crests; less prominent enlarged tubercles on throat, chin and venter; indistinct and smaller dorsolateral tubercles (not reliably countable), and a prominent enlarged pointed tubercle on lateral side of head. Its hemipenes are less well extruded than those of the holotype, and the fleshy apical lobes, although present, are difficult to identify.

Etymology. The species name is a patronym dedicated to Ted Townsend, in recognition of his important contributions to the phylogenetics and systematics of squamates, chameleons, and *Brookesia* in particular.

Conservation status. This species is currently only known from relatively high elevation on the Marojejy massif. We follow assessments for other chameleon endemics from this area on Marojejy, specifically *Calum-*

ma jeje and *C. peyrierasi*, which are found somewhat higher but probably have a similar level of microendemism. We consider the species Vulnerable under IUCN Red List criterion Vulnerable D2: as far as is known, *B. tedi* has a highly restricted area of occupancy (= extent of occurrence) of under 150 km² (this is the area of 1200 m a.s.l. and above in Marojejy), and is known from a single threat-defined location at 1300 m a.s.l. and above in Marojejy National Park. Two plausible future threats, namely decrease in efficacy of protection on Marojejy, and fire, could rapidly drive the species to becoming Critically Endangered.

Table 1. Morphometric measurements of holotype and paratype of *Brookesia tedi* sp. n. (all in mm). See Materials and methods for abbreviations.

	ZSM 438/2016 ZCMV 15262 Holotype	ZSM 439/2016 ZCMV 15298 Paratype
Sex	M	M
TL	29.5	31.7
SVL	15.3	18.2
TaL	14.2	13.5
HW	3.2	3.6
HH	2.7	2.9
ED	1.6	1.8
FORL	5.3	5.3

Discussion

The new species *Brookesia tedi* is a distinctive, new, and comparatively high-elevation member of the *Brookesia minima* species group. Despite being the sister species of *B. peyrierasi*, the two species differ strongly in their hemipenis morphology. Indeed, hemipenis morphology is a particularly valuable taxonomic character in this species group (Glaw et al. 1999), with characters of several members manifesting as novel structures, such as the arrays of spines of *B. peyrierasi* (Fig. 6), single spines of *B. desperata*, symmetrical short papillae of *B. micra*, and the small structures of the apex of *B. tuberculata* (Brygoo and Domergue 1975; Glaw et al. 1999, 2012a). The apical papillae of *B. tedi* are again different, and the hemipenes of this species resemble somewhat those of *B. ramanatsoai* and *B. minima* in their globular construction and apical lobes (Glaw et al. 1999, 2012a). The evolutionary significance of this variability among species of *Brookesia* remains unclear, but it is a promising area for future research.

Brookesia tedi joins the ranks of species from the Marojejy massif—an assemblage that has been growing rapidly in recent years. The majority of the species that have recently been described from Camp Simpona at 1325 m a.s.l. are amphibians (Glaw and Vences 2000, 2011; Glaw et al. 2011, 2012b; Scherz et al. 2014, 2016, 2017; Rakotoarison et al. 2017) and only one is a reptile (Prötzel et al. 2018). Some of these species have shown an interesting biogeographic connection with similar elevations on the Sorata massif, which we have discussed elsewhere (Scherz et al. 2018b). Data from miniaturised frogs of the genus *Stumpffia* suggest that this pattern can occur also in miniaturised species with supposedly low vagility, but most species of that genus show closer relationships with taxa from areas to the south and east of Marojejy (Rakotoarison et al. in press). The close affinities of *B. tedi* with *B. peyrierasi* from Masoala, Nosy Mangabe, and Ambodivoangy conform rather to this latter pattern.

The two type specimens of *B. tedi* showcase the intraspecific variability that can be present in members of the *B. minima* species group. The holotype has only low tubercles on its head and relatively low supraocular cones, whereas the paratype has pronounced spiny tubercles, and an especially large supraocular cone (Fig. 5). Some morphological variability is present in other members of the group as well; characters such as dorsolateral spines can be present or absent within a single species (Glaw et al. 2012a). Substantial variation in several characters also characterizes other, larger-bodied members of the genus *Brookesia*, e.g. regarding the form of the pelvic shield or the number of dorsolateral spines (see Scherz et al. 2018a for an extreme example). However, there are no *Brookesia* outside the *B. minima* species group where the dorsolateral spines can be present or absent. It is probable that the miniaturisation of this group has resulted in increased morphological variability of adults, as is often seen in miniaturised animals (Hanken and Wake 1993).

Apart from Montagne d'Ambre, at present, Marojejy is the only other known locality where two species of the *B. minima* species group occur. Despite this, and similar to the situation on Montagne d'Ambre, *B. tedi* and *B. karchei* possibly do not occur syntopically, the former being known from at and above 1300 m a.s.l. and the latter from under 1000 m a.s.l. However, still unidentified *Brookesia minima*-group specimens are known from lower altitudes of Marojejy (Raxworthy and Nussbaum 1995; Raselimanana et al. 2000) and future studies need to clarify their taxonomic status. Other populations from the vicinity of Sambava (Glaw and Vences 1994) must also be investigated, as these may represent further undescribed diversity within this group.

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